fragmentation of partially decorated filaments at boundaries of bare and cofilin-decorated segments. Despite extensive evidence for cofilin-dependent changes in filament structure and mechanics, it is unclear how the two processes are linked at the molecular level. Here, we use molecular dynamics (MD) simulations and coarse-grained (CG) analyses to evaluate the molecular origins of the changes in filament compliance due to cofilin binding. Filament subunits with bound cofilin are less ''flat'' and maintain a significantly more open nucleotide cleft than bare filament subunits. Decorated filament segments are less twisted, thinner (considering only actin), and less connected than their bare counterparts, which lowers the filament bending persistence length and torsional stiffness. using coarse-graining as an analytical method reveals that cofilin binding increases the average distance between the adjacent long-axis filament subunit, thereby weakening their interaction. In contrast, a fraction of lateral filament subunit contacts are closer and presumably stronger with cofilin binding. These results reveal the molecular origins of cofilin-dependent changes in actin filament mechanics that promote filament severing.

2823-Plat
Formin mDia1 is a Mechano-Sensor that can Tense Actin Filaments
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The study of actin assembly dynamics at the scale of individual filaments provides key information on the molecular mechanisms at play. using microfluidics, we have developed experimental configurations which provide a straightforward and accurate monitoring of individual filaments in vitro, with an extensive control of their biochemical environment (Jégou et al. PLoS Biology 2011, Niedermayer et al. PNAS 2012). Here, we use our microfluidics setup to investigate how a formin responds to mechanical tension exerted on the actin filament it is elongating. This situation is encountered in living cells, and by studying it in vitro we also gain insight into molecular details of formin activity. In our experiments, the applied force results from the viscous drag exerted by the flowing fluid on the actin filament, and mDia1 (FH1-FH2) formins are anchored either to the bottom of the flow cell, or to a bead held in an optical trap for direct monitoring of the force. By specifically anchoring formins via their FH1 or FH2 domains, we can pull on the FH2 domain alone, or stretch the FH1 domain as well. By measuring how formin activity is affected by picoNewton forces stretching their FH1 domains, we get new insight on the ''capture and delivery'' mechanism responsible for rapid elongation from profilin-actin. By pulling on the FH2 domain of the formin dimer, we obtain new information on the translocation of the FH2 dimer during processive elongation. We also measure the pulling forces generated by formins bound to depolymerizing actin filaments, showing that formins can put filaments under mechanical tension.

2824-Plat
Mechanical Properties of Individual Actin Crosslinks
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The internal organization of eukaryotic cells is provided by the cytoskeleton, a highly dynamic protein network mainly composed of actin filaments, microtubules and intermediate filaments. The mechanical and dynamical properties of this network are not simply given by the filament mechanics, but can be modulated specifically by various crosslinking and motor proteins. The characteristics of these crosslinked actin networks have been studied extensively in ensemble experiments, while detailed information on crossing-overs of single filaments is still rare.
We developed a four-bead optical tweezers assay to probe mechanical properties of individual, freely suspended actin-crosslinker-actin bonds. We then compared the forced unbinding of the crosslinking proteins filamin A and alpha-actinin 2 which share a high similarity in their actin binding domains. Interestingly, our single-molecule unbinding assay reveals a significantly different behavior: An increase in force results in faster unbinding of alpha-actinin-crosslinks ('slip bond'), however, the opposite is true for filamin ('catch bond'). We further used genetically engineered variants of the crosslinkers to investigate the molecular mechanism underlying this significant difference in the mechanical unbinding behavior of these crosslinking proteins.

2825-Plat
Myosin Activity Drives Cytoskeletal Networks to a Critically Connected State
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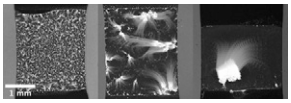
The cytoskeleton is a fascinating material, where myosin pulling forces drive actin networks out of equilibrium. Recent studies have found that network response to motor activity is governed by connectivity: weakly connected networks give rise to ordered patterns and dynamic clusters, whereas well connected networks are elastic and propagate tension. Theory has predicted critical intermediate states between these two regimes, but experimental evidence for such states remains elusive. Here we experimentally study motor-driven activity in in-vitro actin networks over a broad range of network connectivities. We show the network contracts into clusters that exhibit a scale-free distribution of sizes, characteristic of a critical state. Surprisingly, this critical behavior occurs over a broad range of network connectivities. To explain this robustness, we perform simulations of contractile networks taking into account network restructuring: motors can reduce connectivity by promoting crosslink unbinding. We demonstrate that this coupling between activity and connectivity drives initially well connected networks to a critically connected state. Furthermore, we propose that recent examples of cytoskeletal rupture in cells and tissues can be simply explained by network restructuring, which controls the length scale of contraction (see figure).



2826-Plat
XMAP215 and EB1 act in Synergy to Promote Microtubule Growth
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In cells, a complex network of microtubule-associated-proteins regulates the dynamic growth and shrinkage of microtubules that is essential for division and migration. In vitro approaches with purified components have helped to elucidate the mechanisms and the effects of individual microtubule plus-end-localizing proteins (+TIPs) on microtubule dynamics. Because microtubule dynamics observed in vitro with individual +TIPs does not account for the dynamics observed in vivo, it is important to study the combined effects of +TIPs. Here we show that two well-studied +TIPs - microtubule plus-end-tracking protein EB1, and the microtubule polymerase XMAP215 - act together to strongly promote microtubule growth to rates never before observed with purified proteins. Unexpectedly, we find that the combined effects of XMAP215 and EB1 are highly synergistic, with acceleration of growth well beyond the product of the individual effects of either protein. The synergy remains after EB1's C-terminal 20 amino acids have been removed, showing that it does not rely on any of the canonical EB1 interactions. The increase in growth rates is accompanied by a strong enhancement of microtubule catastrophe, thereby rendering the fast and dynamic microtubule behavior typically observed in cells.

2827-Plat
Microrheology of Highly Crosslinked Microtubule Networks is Dominated by Force-Induced Crosslinker Unbinding
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We determine time- and force-dependent viscoelastic responses of reconstituted networks of microtubules that have been strongly crosslinked by biotin-streptavidin bonds. To measure the microscale viscoelasticity of such networks, we use a magnetic tweezers device to apply localized forces. At short time scales, the crosslinked networks respond nonlinearly to applied force, with stiffening at small forces and softening at high forces, which we attribute to the force-induced unbinding of crosslinks. At long time scales, force-induced bond unbinding leads to local network rearrangement and significant bead creep. Interestingly, the network retains its elastic modulus even under conditions of significant plastic flow, suggesting that crosslinker breakage is balanced by the formation of new bonds. To better understand this effect, we developed a finite element model of such a stiff filament network with labile crosslinkers obeying force dependent Bell model unbinding dynamics. We confirm that for rigid MT filaments having many crosslinks, the coexistence of dissipation and elastic recovery of the network is possible as a result of bond unbinding and rebinding events. Elastic recovery can occur as long as a sufficient number of the original crosslinkers are preserved under the loading period. Plastic flow increases with the decreasing fraction of original